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Technical Memorandum M-109

THE ROLE OF *Limnoria tripunctata* IN PROMOTING EARLY FAILURE OF CREOSOTED PILING

6 April 1956

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port hueneme,
california

U. S. Naval Civil Engineering Research and Evaluation Laboratory
Port Hueneme, California

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THE ROLE OF *Limnoria tripunctata* IN PROMOTING EARLY FAILURE OF CREOSOTED PILING

6 April 1956

H. Hochman, H. Vind, T. Roe, Jr.,
J. Muraoka, and J. Casey

SUMMARY

Heavy impregnation with coal-tar creosote usually protects wooden piles in temperate waters from marine borer attack. However, even the best creosoting practices are often ineffective in tropical and subtropical waters. The difference between the species of marine borers prevalent in tropical waters and those in temperate waters was considered as a possible explanation for the differences in serviceability of treated timbers. Evidence was obtained which supports the postulate that a single species, *Limnoria tripunctata*, initiates the failure of creosoted piling in California harbors. This species inhabits tropical and subtropical waters only.

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INTRODUCTION

Destruction of wooden harbor installations by marine boring organisms is a problem of great economic importance the world over. Many methods have been employed to protect timbers from the deteriorating action of these boring organisms, the most popular and probably most successful method being impregnation of the timbers with coal-tar creosote.

The effectiveness of coal-tar creosote as a marine borer deterrent varies considerably. Although heavy impregnation with coal-tar creosote usually gives long life to piles in temperate waters, even the best creosote treatments are often ineffective in tropical and subtropical waters. In temperate waters treated piling quite generally has a service life of more than twenty years, whereas in tropical waters ten years is a more typical service life, and in some harbors creosoted piling fails in less than ten years. In addition to these geographical differences, the effectiveness of creosote as a preservative depends upon the condition of the surface of the treated timber. The protection afforded to sawed timbers is less satisfactory than that afforded piles. If the reasons for these differences in the effectiveness of creosote treatment were known, ways in which to improve the treatment or treating agent could be more readily developed.

Historical Foundations

Hunt, in 1948 stated that a factor of importance in determining the service life of creosoted piling is the species of marine borers present in the harbor.[1] He pointed out that the best creosote treatments are often ineffective in tropical waters heavily infested with *Limnoria* and thus inferred that it is *Limnoria* and not the teredine borers or shipworms which lead to the failure of creosote treatment.

In 1952 Menzies discussed the reasons for failures of creosote to protect harbor pilings from marine borer attack.[2] The most often discussed causes of these failures are the use of inferior grades of creosote, inadequate impregnation procedures, and improper methods of dock construction such as notching or drilling bolt holes beyond the depth of creosote penetration. Menzies further pointed out that even when such factors are not involved, marine borers frequently destroy creosoted timber in warm waters long before similarly treated timber is destroyed in colder waters.

On plotting the geographical distribution of the harbors in which early failure of creosoted piling had been reported, he noted that the distribution coincided with the distribution of the species of marine boring organisms, *Limnoria tripunctata* . He then examined test blocks of creosoted wood taken from several of these harbors. The only species of marine boring organisms which he could find was *Limnoria tripunctata* . The species *Limnoria tripunctata* was first described in 1951 by Menzies who discovered them in the San Diego harbor.[3]

Statement of the Problem

Menzies inferred that the species *Limnoria tripunctata* initiates the failure of creosoted piling in California harbors and other southern ports. It was desired to prove or disprove this contention. Well-confirmed results attributing the initiation of the deterioration of creosoted piling to a single species would have an important bearing upon the direction of future marine borer research. Such results would also have a bearing upon the selection of criteria by which to evaluate the usefulness of various brands of creosote and creosote additives to be employed in the treatment of piling for southern ports.

The harbor at Port Hueneme, California, is an ideal harbor in which to test the hypothesis that the species *Limnoria tripunctata* initiates the deterioration of creosoted piling. *Limnoria tripunctata* are present in substantial numbers but they are not the principal species of *Limnoria* in the harbor. *Limnoria quadripunctata* are more abundant. *Teredo, Chelura,* and *Bankia* are also present in large numbers.

The piling of Wharf No. 3 at Port Hueneme was installed in the summer of 1942 as a temporary dock with an expected life of ten to fifteen years. No dock had existed at this site previously.

The firm of Atkinson-Pollock of Long Beach were the contractors. The specifications called for all piling and bracing to be of select structural-grade Douglas Fir and to be creosoted by a 16-lb full-cell process with grade No. 1 creosote oil.

Wharf No. 3 in the harbor at Port Hueneme began to be expanded and rebuilt in June of 1955. The old piling, which had been in the water for more than ten years, is being pulled and can therefore very conveniently be examined for evidences of marine borer attack so that there is available for study at the U. S. Naval Civil Engineering Research and Evaluation Laboratory creosoted piling which has been attacked by marine boring organisms.

It was planned simply to assess the extent of damage to these piles and to determine which species of marine borers were causing the damage. It was also planned to make limited observations of the extent to which creosote had been leached from the piling.

EXPERIMENTAL

Field Inspection

The first row of piles was examined as it was pulled from the water. Figure 1 shows the equipment used for pulling the piles. At the position designated by the arrow in Figure 1 the pile being suspended by the crane is beginning to assume an hourglass shape. This is typical of piles damaged by *Limnoria*. Figure 2 shows the inspection team at work. Several of the connecting or bracing members were completely destroyed, as is the one shown in Figure 3. Several of the piles had deteriorated to the extent that they were nonfunctioning but most of them still were in fair condition. As will be discussed later in this report, in the ensuing years the piling would have deteriorated at a greatly accelerated rate.

Those regions which were covered with fouling organisms were virtually free of attack. This was true even when the covering was light. The several feet of piling just above the mud line and those in the intertidal zone were free from fouling organisms. In these two regions *Limnoria* attack was heavy and one-half to one inch of the creosoted layer had been destroyed.

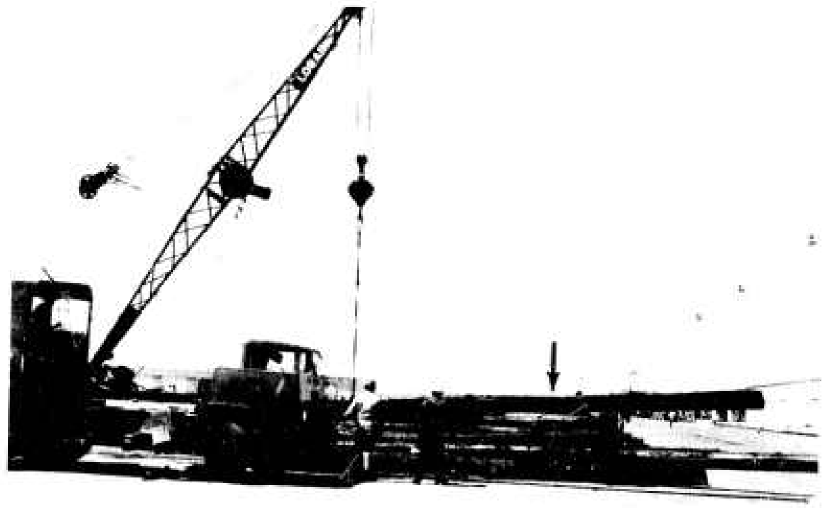

Figure 1. Equipment employed in removing old piling from the harbor.

Figure 2. Inspection team examining piling removed from the harbor.

Figure 3. Creosoted whaling log which has succumbed to *Limnoria* attack.

The attack was not confined to bolt holes, broken spots on the pile, and the like, as is often claimed, but was also on the open, still heavily creosoted face of the piling. Figure 4 presents a close-up view of the surface of a pile and discloses heavy *Limnoria* attack of wood still oozing with creosote. The figure clearly shows *Limnoria* crawling on and burrowing into the creosoted wood. Chips removed from the surface and brought into the laboratory for further study contained sufficient creosote to fill the room with the distinctive creosote odor and to stain the fingers of those examining the chips.

There appeared to be much greater activity in the movement of the *Limnoria* from their burrows to the surface of the wood and back again into the burrows than had been previously noted for *Limnoria* attacking untreated wood. This behavior may be necessary for survival of *Limnoria* in the presence of creosote.

At the intertidal zone the diameter of the piles is reduced both by the action of *Limnoria* and the action of the "camel logs" (fender logs floated in front of the dock to keep vessels from direct contact. The camel logs, which float on the surface of the water in front of the first tier of piles, rub back and forth against the surface of the piling with the action of each wave and each tide. This process wears a flat or smooth area on each pile in the intertidal zone. Erosion from this source is most extensive at the center of the intertidal zone and tapers off toward the upper and lower extremes of the intertidal zone. Figure 5 illustrates such an area. In most cases the camel logs had worn through the apparent depth of creosote penetration. However, colorless compounds in the creosote may penetrate more deeply than would be judged by the black areas of the wood. Wood removed from those areas worn smooth by the action of the camel logs still retained a distinctive creosote odor. No indication of *Teredo* or *Bankia* attack was noted. *Limnoria* attack was also light on these surfaces. It is possible that the constant rubbing of the camel log against the piling served to mechanically kill most of those attempting to lodge on this surface. Opposite the surface worn smooth by the camel log was the area of the wood most heavily attacked by *Limnoria*. The combined action of these two processes was serving to restrict the diameter of the pile to produce an hourglass contour.

In the region just above the mud line was another region of heavy *Limnoria* attack. Attack at this level was not as severe as that in the intertidal zone and near-intertidal zone. Nevertheless, because

Figure 4. *Limnoria* crawling and burrowing on the surface of creosoted piling.

Figure 5. Surface of a pile worn smooth by the action of the "camel log."

the piles are driven small end down, moderate attack at this level is as dangerous to the safety of the pier as heavier attack is at the intertidal zone.

Figure 6 shows the clear line of demarcation at the mud line. Below the mud line the piles were untouched and here the surface was still "wet" with creosote (see Figure 7). There were a few *Limnoria* burrows extending down below the line of demarcation and into the mud zone for a distance of three to six inches. Below that, however, there was no attack whatsoever nor was there any sign of fouling or mold growth. The occurrence of a few *Limnoria* in the first several inches below the mud line was interesting and might indicate a shifting mud line. Though the burrows were easy to find, living *Limnoria* were found only after several minutes of searching. These were living and yet were essentially swimming in creosote.

Several cross-sectional cuts were made at various locations on the timbers. Figures 8 and 9 illustrate two of these cuts. From an examination of the cross-sectional areas, estimates of the extent of creosote penetration and the extent to which the layer of creosote penetration had been eaten away could be made. A further purpose was to examine the heart of the piling for evidences of teredine borer attack. None could be found. Attack was restricted to the outer inch of exposed surface. Apparently the piling had originally been creosoted to a depth of about one or two inches from the surface. At the points under heaviest attack, at least half of this layer had been stripped. In the intertidal zone the camel logs had completely worn through the layer of creosote penetration on one side of each piling. Finally, the cross-sectional cuts gave the impression that creosote diffuses inward by the action of sea water as well as being leached from the piling.

Examination of those areas covered by fouling organisms revealed very light attack. Figures 10 and 11 show the examination of the surface of a piling under one of the fouling organisms. The fouling organism, a rock oyster, had given complete protection to the pile. The surface of the pile underneath the fouling organism appeared like new. Even in those regions covered only lightly by a thin coating of Bryozoa the attack was very light.

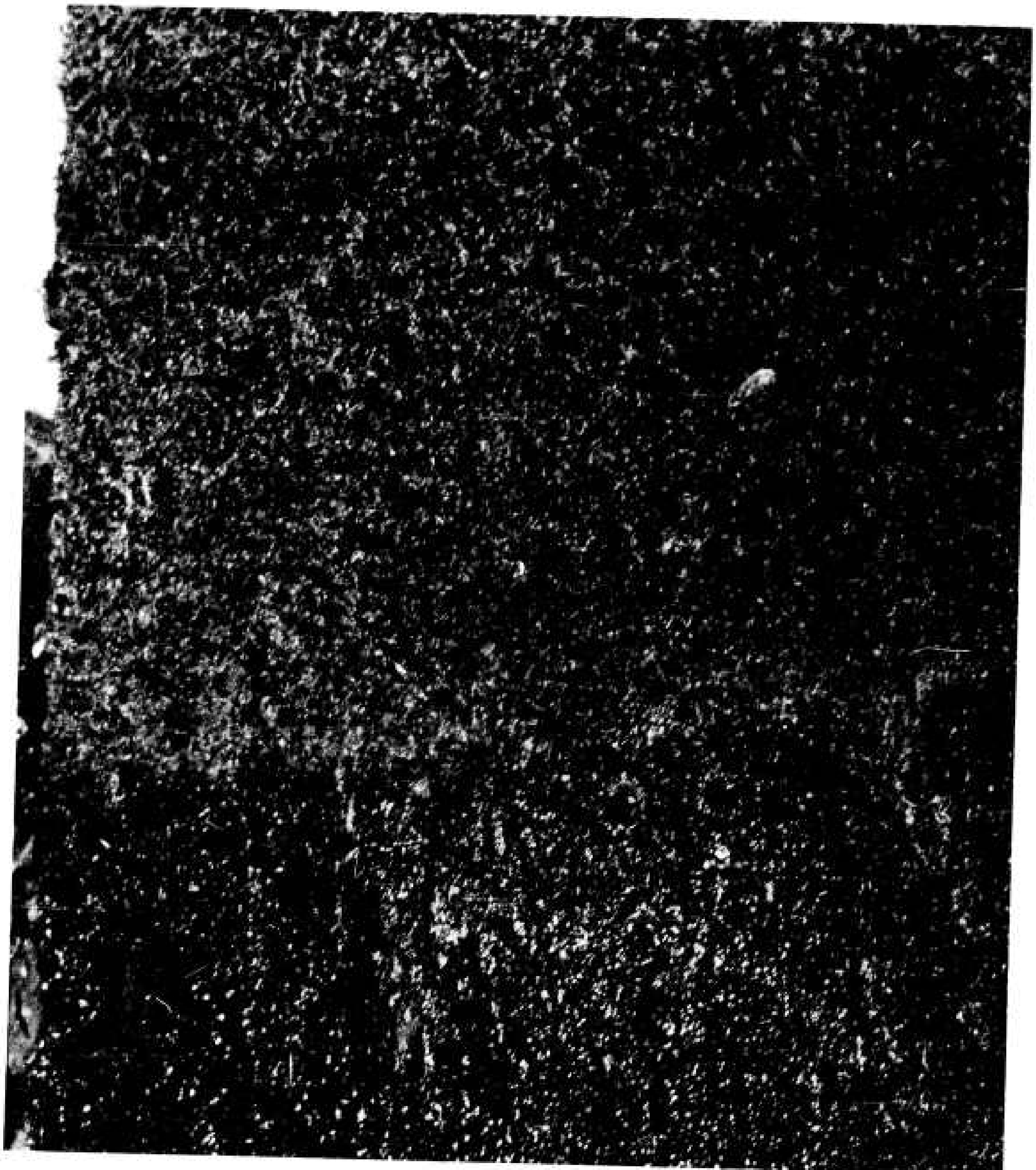

Figure 6. Reduced *Limnoria* damage below the clearly defined mud line.

Figure 7. Surface of a pile from below the mud line.

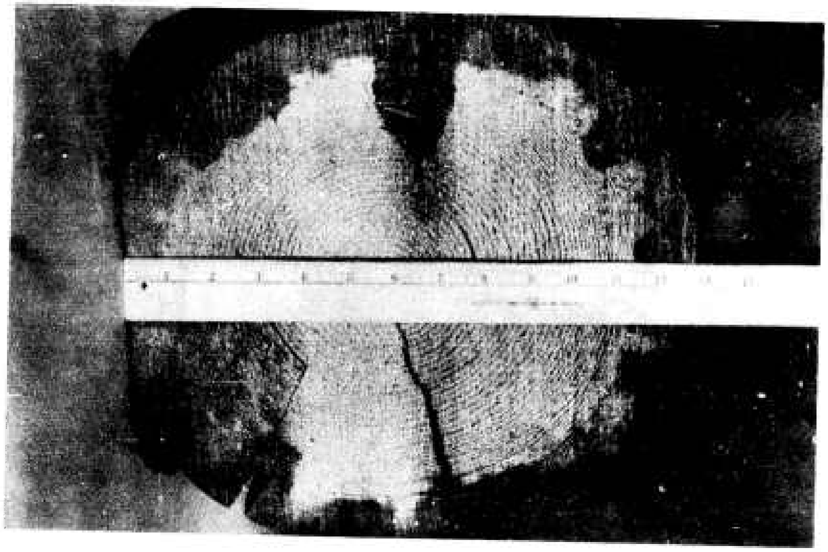

Figure 8. Cross section of a pile from above the water line, illustrating the original depth of creosote penetration.

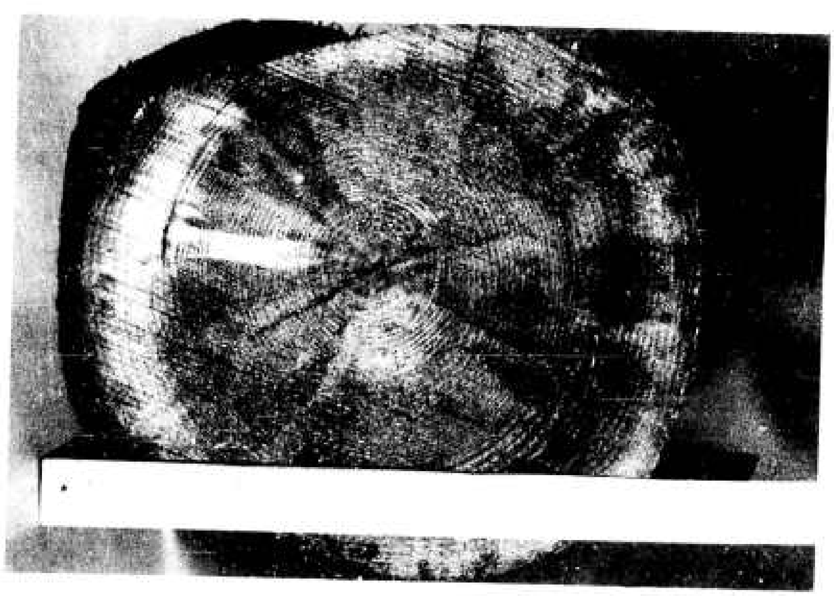

Figure 9. Cross section of a pile from the intertidal zone, illustrating the extent to which the creosoted layer has been dissipated or destroyed.

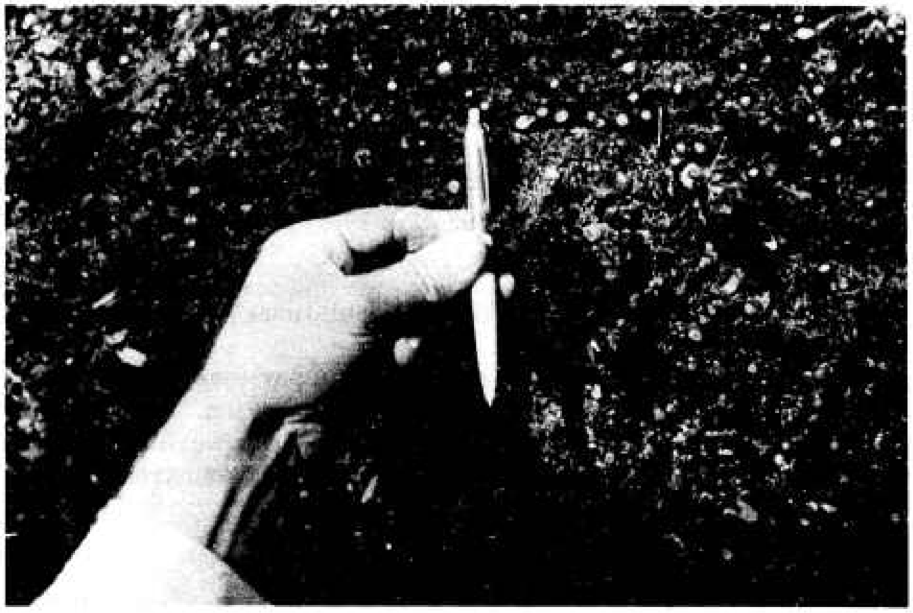

Figure 10. Examination of a rock oyster fouling the surface of a pile.

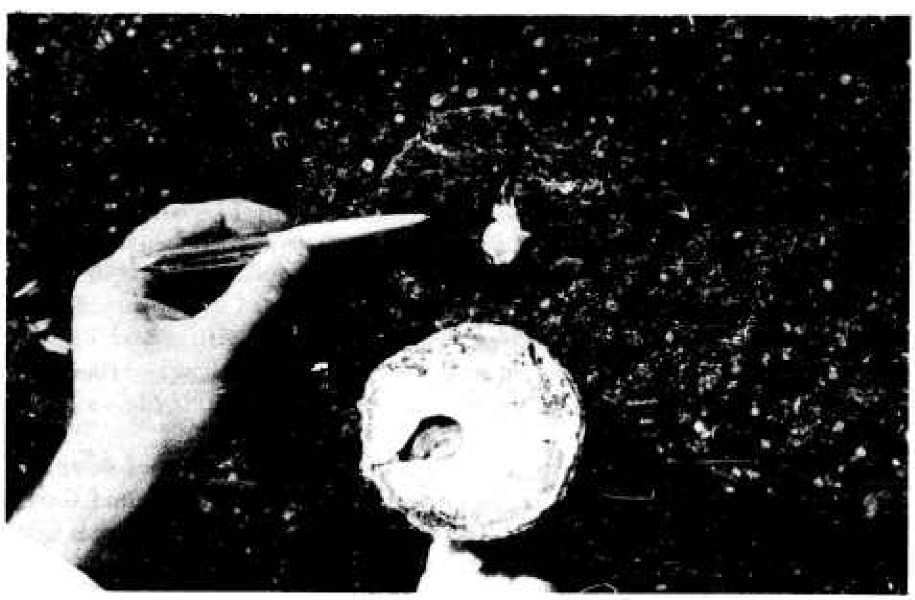

Figure 11. Examination of that portion of the surface of a pile which had been protected by a rock oyster.

In the field examination no evidence of *Bankia* or *Teredo* attack was found. The only species of marine borers to be found were *Limnoria* and *Chelura*. The *Chelura* were uncommon and their occurrence was spotty. *Chelura* are considered by many to be secondary invaders only. The distribution of *Limnoria* appeared to be more uniform and more widely spread.

Laboratory Studies of Marine Borer Populations

Chips or slabs removed from various portions of piling were brought into the laboratory for detailed examination. The objectives were to determine what species of marine borers were attacking the wood and, if possible, to determine why the creosote treatment had failed to protect the wood from borer attack.

Estimating the Marine Borer Population of Creosoted Piling

Slabs were removed from the piling with a hand ax. They typically measured about one foot in length, six inches in width, and one or two inches in thickness. These were laid in trays containing an inch or two of sea water and brought into the laboratory. Some *Limnoria* left the blocks before a count of the invading organisms had been made. The figures obtained were thus lower than the true population of animals on the piling.

Preliminary examination revealed that for practical purposes the wood harbored only the species *Limnoria tripunctata*. Chips removed from heavily attacked regions of the wood also harbored appreciable numbers of an unidentified species of *Chelura*. Figure 12 illustrates the *Limnoria* removed from creosoted piling and Figure 13 the common species which collects on untreated piling. *Chelura* removed from creosoted piling are pictured in Figure 14.

Counts of invading organisms were made on each of several small chips. These chips were completely picked apart and the marine borers were collected and segregated into the various species. Table I is a summary of the results.

In those regions under attack, the *Limnoria* density was certainly greater than 100 per sq in. of piling surface. As already discussed, the counts obtained on the chips tended to be lower than the actual

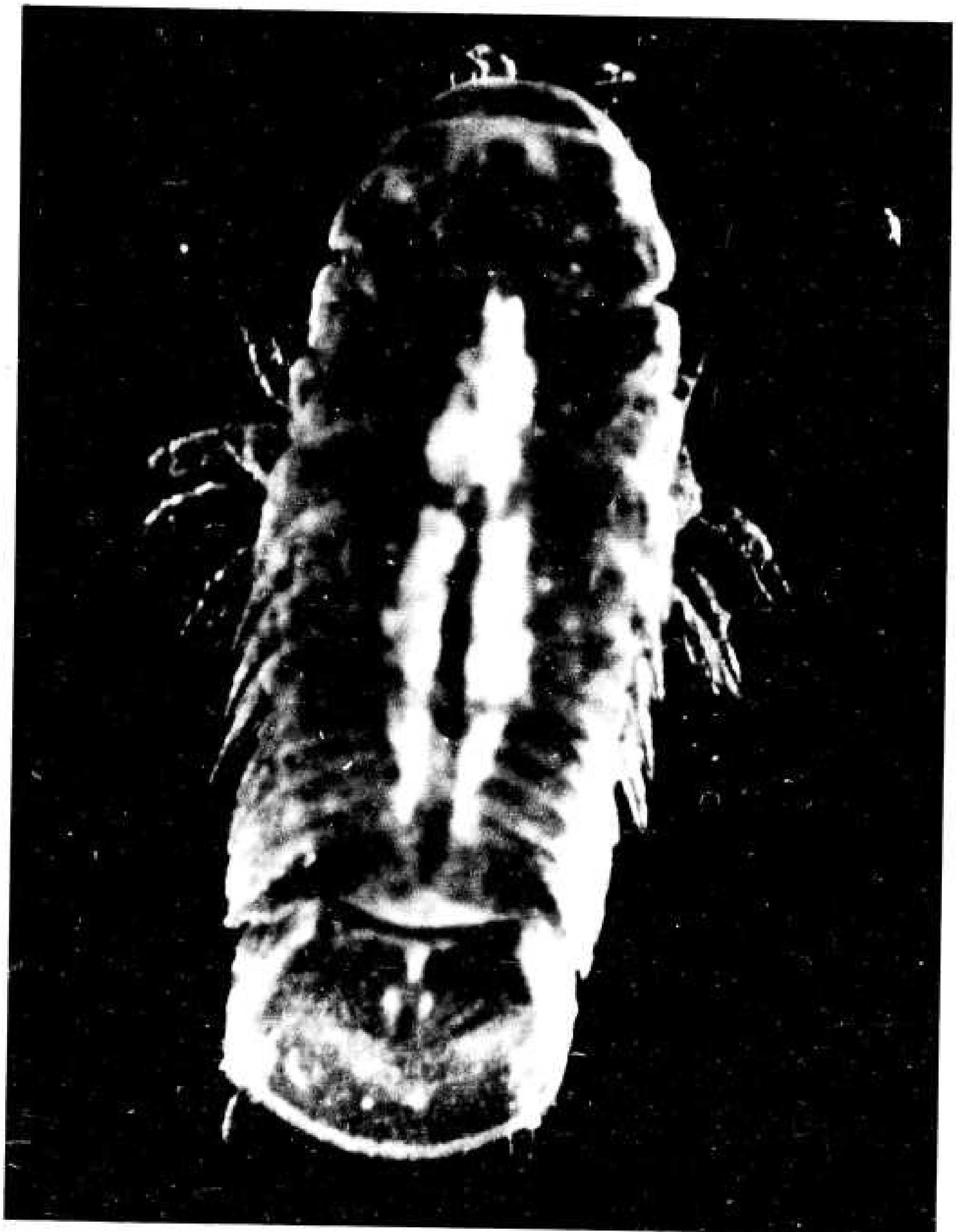

Figure 12. Typical marine borer found on creosoted piling and identified as *Limnoria tripunctata*.

Figure 13. Common species of *Limnoria* found on untreated test blocks at Port Hueneme and identified as *Limnoria quadripunctata*.

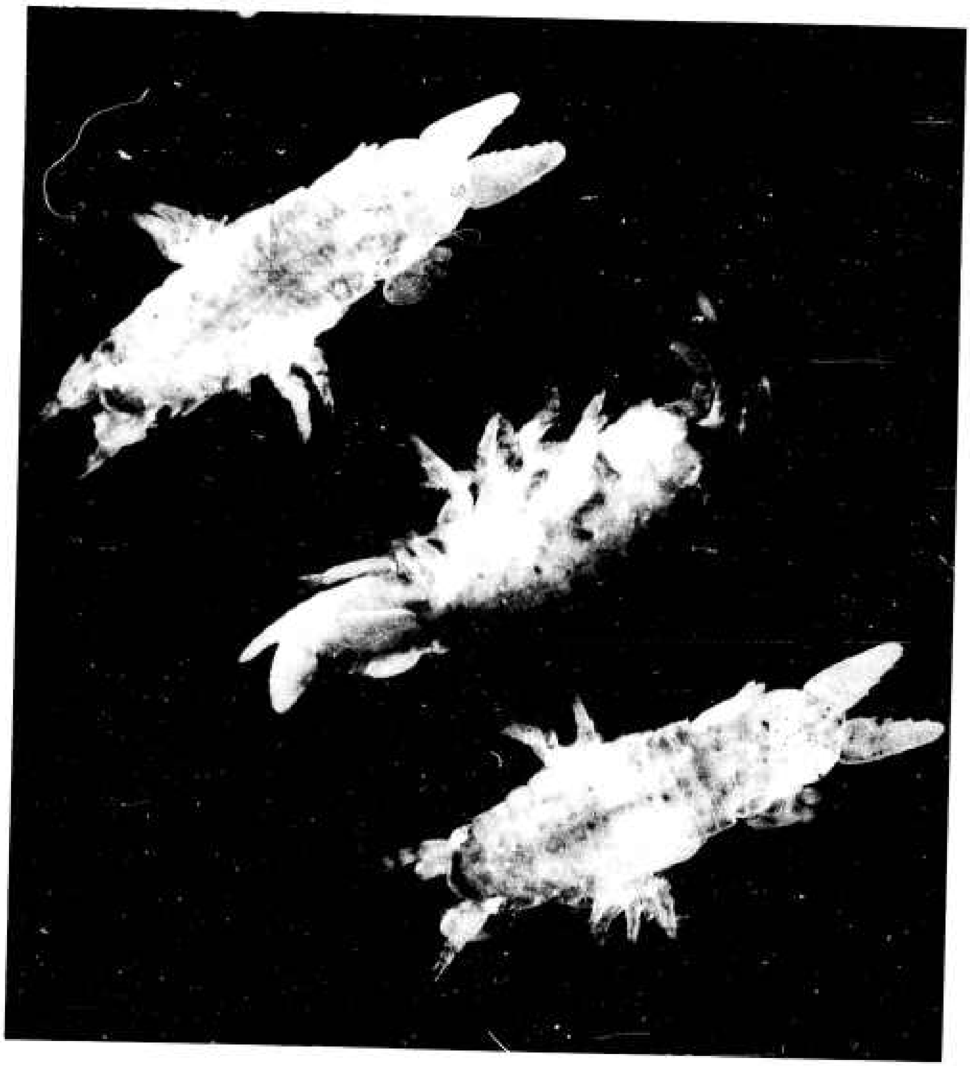

Figure 14. *Chelura* removed from creosoted piling.

TABLE I. Number of marine borers found on chips removed from the surface of creosoted piling.

Location on piling	Organism and number found		Population per square inch
1. Intertidal zone	*Chelura* *L. tripunctata* *L. quadripunctata*	100 607 12	238
2. Just below intertidal zone	*Chelura* *L. tripunctata* *L. quadripunctata*	0 310 0	207
3. Just below intertidal zone	*Chelura* *L. tripunctata* *L. quadripunctata*	5 298 0	151
4. Near mud line	*Chelura* *L. tripunctata* *L. quadripunctata*	0 225 0	228
5. Fouled region midway between water line and mud line	marine borers (However, a few *Limnoria* tracks were noted.)	0	0

population density of the pilings. The true *Limnoria* density on the pilings was probably closer to 300 per sq in.

Number of *Limnoria* Collected on Untreated Test Blocks

In order to obtain animals for toxicity studies at NAVCERELAB, laminated blocks of pine or fir measuring 4 in. by 4 in. by 12 in. were bolted to channel iron racks and these racks were suspended horizontally in the harbor about two feet from the mud line. The test pier is located in the same harbor as that from which the creosoted piling had been obtained. After a period of several months the racks were pulled out of the waters and the test blocks removed and stored in tall narrow jars (pipette cleaning jars) of sea water. Figures 15 and 16 illustrate a rack of test blocks before and after an exposure of eight months in the harbor.

Every morning for several consecutive mornings each block was removed from its storage jar and the *Limnoria* crawling on the surface were transferred to small Petri dishes of sea water. The blocks were then returned to the jars of sea water and were stored overnight at about 40 F. Cooling and standing seems to induce the borers to crawl to the surface. Results of experiments in progress suggest that carbon dioxide tension may be the actual stimulus which induces them to crawl out of their burrows. After one week very few *Limnoria* were left on the block.

The purpose of these collections has been to obtain animals for toxicity tests and not to determine the population of borers on the test blocks. Only living adult *Limnoria* have been collected. The true population of *Limnoria* on the test blocks may have been two or even three times greater than the numbers of animals collected. Nevertheless, the number of animals collected from each block gives an idea of the order of magnitude of the population on that block. These block populations are reported in Table II. Surface area of each block was very close to one square foot.

Figure 17 illustrates the extent of *Limnoria* damage to the surface of one of the laminated test blocks. Figure 18 presents a magnified view of the surface of the same block. The pitted surface clearly demonstrates that *Limnoria* were attacking the test block. Yet, the attack by such a sparse population of *Limnoria* as had developed in

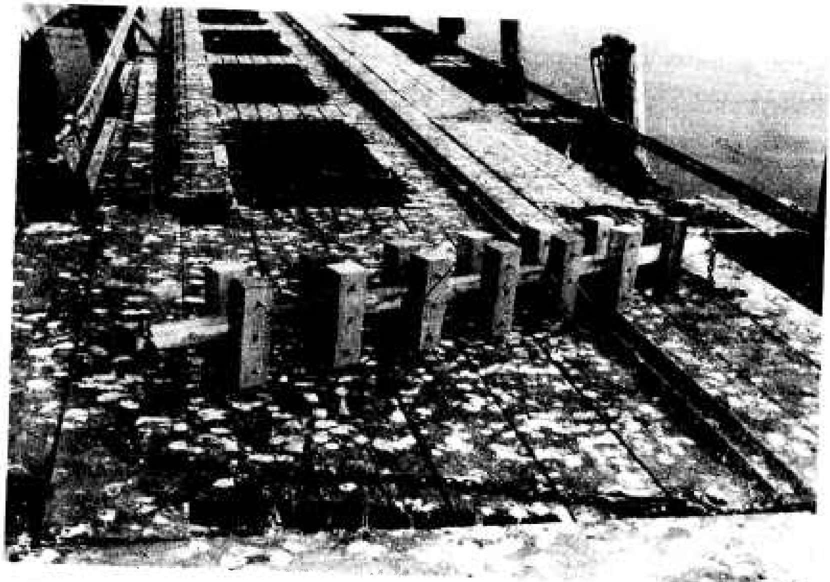

Figure 15. Rack of collecting blocks ready to be placed in the harbor.

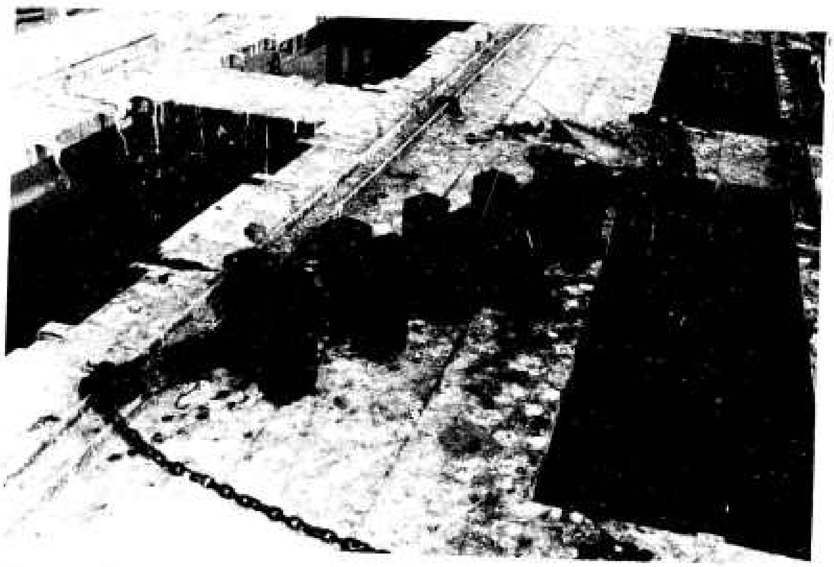

Figure 16. Rack of collecting blocks immediately after being removed from the harbor. (Time in the harbor, eight months.)

TABLE II. Number of *Limnoria* removed from untreated test blocks.

Rack	*Limnoria* per block	Months in the water
40	152 217 874 451 466 566	8
43	43 40 33	9 (all blocks heavily fouled)
41	635 591 567 866 722	8
*34	1500 (approximate) 2000 (approximate)	11

*An experiment in which 3000 animals were employed was performed from the *Limnoria* removed from these two blocks. No actual count had been made of the animals removed, however.

Figure 17. Surface of an untreated test block lightly pitted from *Limnoria* attack.

Figure 18. Close-up view of the surface of an untreated test block lightly pitted from *Limnoria* attack.

eight months' time was too light to cause extensive damage to the blocks. The extent of the *Teredo* and *Bankia* attack, however, which occurred in eight months was not light, as can be seen in a cross section of the same block illustrated in Figure 19. Infestation by these animals can occur quite suddenly.

Two dishes of *Limnoria* were taken at random from the supply which had been removed from the blocks on Rack No. 41. The *Limnoria* in the two dishes were examined individually under a wide-view microscope. The *tripunctata* were separated from the *quadripunctata*. In the first dish there were 96 *tripunctata* and 55 *quadripunctata*. In the second dish there were 36 *tripunctata* and 55 *quadripunctata*. These figures are typical of the relative distribution of the two species on the collection blocks examined during the past several months and is perhaps indicative of the relative abundance of the two species in this harbor. However, a definite statement concerning their relative prevalency in these waters could not be made without more data on collections made throughout all seasons of the year and all parts of the harbor.

Extent of *Limnoria* Damage to Untreated Test Chips

Ten *Limnoria tripunctata* were placed upon presoaked pine chips measuring 1 in. by 2 in. by 1/8 in. The chips were suspended in dishes of sea water on shelves made of plastic screening material. After two months the damage to the chips was noted. Numerous tunnels had been bored throughout most of the length of the chips. Several holes penetrated completely through each chip. The extent of the boring can be seen in Figure 20.

Discussion

Atwood and Johnson[4] in a general discussion of *Limnoria* stated, "A single square inch of timber may contain 300 to 400 animals of all ages." The inference was that this is the maximum density that is attained. The population density of *Limnoria* on the creosoted piling, therefore, was perhaps near to the maximum attainable. The borer population of the untreated collecting blocks, which had been in the water for eight or nine months, was at most only a few per cent as dense as that of the creosoted pilings, which had been in the ocean for thirteen years.

Figure 19. Cross section of a collecting block which had been in the harbor for eight months.

Figure 20. Chip of untreated pine after three months' exposure to ten *Limnoria*.

In the same discussion Atwood and Johnson stated, "The young, when hatched, differ only in size from the adults and are ready to bore at once, and they begin their work near the parent, so that an infestation generally spreads slowly from a center."

A general pattern of invasion by *Limnoria* begins to emerge. It would appear that heavy infestation does not occur suddenly as it often does with *Teredo* or *Bankia* . A few *Limnoria* migrate to the piling from other breeding places and the piling now becomes their new site of breeding. There ensues a period of several years of steady growth in population. Each female *Limnoria* that breeds produces six to seventeen young in a single brood. They probably breed at least once each year, but to the best of our knowledge the number of breedings per year has not been established.

This pattern of gradual infestation may not be typical of all harbors. The migration of *Limnoria* from other breeding places no doubt occurs very rapidly in some tropical harbors. At Pearl Harbor, for example, even creosoted piling has been reported to suffer severe damage from *Limnoria* within a period of a few years.

This is in contrast to *Teredo* or *Bankia* which in a single reproductive period produce thousands of larvae. Should the larvae find a wooden structure suitable for boring, infestation will be very heavy in a single season. Many untreated piles have failed in less than one year's time because of teredine borer attack.

During the first several years or so of *Limnoria* infestation the population of *Limnoria* probably does not exceed 10 to 50 per sq in. Evidence of boring is observable but the damage is not of economic importance. In later years, when the population reaches several hundred per sq in., damage is severe. It is not likely that piling would completely fail as the result of *Limnoria* attack alone in much less than ten years. Although the damage to the creosoted piling of Wharf No. 3 at Port Hueneme had not in general been severe up to the time that it had been removed from the water, it is likely that during the next several years damage would have been much more extensive.

Toxicity Tests

The toxicity of creosoted piling to marine borers was tested. Chips or splinters of wood removed from the piling were placed in small dishes of sea water and exposed to various marine borers.

Comparative Toxicity of Splinters from Creosoted Piling to Two Species of *Limnoria*.

In each of three dishes of sea water one splinter of untreated presoaked pine and one of creosoted piling obtained from areas adjacent to *Limnoria* burrows were placed. Twenty *Limnoria quadripunctata* from a noncreosoted test block were placed in the first dish. Into the second dish were placed twenty *Limnoria tripunctata* also obtained from the untreated test block. Into the third dish were placed twenty *Limnoria tripunctata* removed from creosoted wood.

After twenty-four hours, fourteen of the twenty *quadripunctata* were dead whereas all of the *tripunctata* were living. The *tripunctata* which had been removed from the noncreosoted wood showed a greater preference for the untreated splinter than for the splinter of creosote-treated wood. The *tripunctata* removed from the creosoted piling appeared at first to be unable to distinguish between the two splinters but distributed themselves evenly between them. One month later the *Limnoria tripunctata* in both dishes were still living. The untreated splinter in both dishes had been completely eaten and the *Limnoria* were burrowing on the treated splinter.

Toxicity of Residual Creosote

The *Limnoria* removed from the surface of creosoted piling did not appear to be tolerant to the creosote from all portions of the piling. A chip or slab removed from the side of a piling was placed in a 5-gallon aquarium with sea water. The dimensions of the slab were approximately 12 in. by 3 in. by 1-1/2 in. Only the outer one-half inch of wood was infested with *Limnoria*. A stream of air was constantly bubbled through the water of the aquarium. Soon the color of the water darkened because of the creosote. Although the water was changed each day, none of the *Limnoria* lived for more than two or three days. On the other hand, living *Limnoria* can be maintained on similar slabs for a week or two if the slab is merely kept covered with wet or damp towels or indefinitely if the slab is maintained in running sea water.

Toxicity of Splinters from Different Areas of the Piling

Splinters of wood were removed at various distances from the exterior of a creosoted pile and the susceptibility of the splinters to *Limnoria* attack was studied.

A 2-in. cross section was removed from a creosoted pile heavily infested with *Limnoria*. The cut was taken a foot or two above the low-tide line. This is the same cross section as shown in Figure 9. A thin wedge extending nearly to the center was cut from it. The wedge taken from this cross section is shown in the upper half of Figure 21.

A similar wedge from a cross section that had been cut above the water line was removed. This is the cross section shown in Figure 8. The wedge from it is shown in the lower half of Figure 21.

For each wedge the approximate original position of the outer edge of the pile is designated by the letter A, the center of the pile by the letter E. The same positions are also indicated by 0 and 6-1/2 inches on the accompanying scale.

A portion of each wedge was dark in color as a result of the creosote. This creosoted portion is designated by the letter B in Figure 21. The creosote zone of the lower wedge is quite sharply defined. The wedge was from a portion of the timber exposed only to the leaching action of rain and not to the leaching action of sea water. The wedge illustration in the upper half of Figure 21, shows no sharp boundary between the creosoted and noncreosoted areas. In this case sea water had caused the creosote to diffuse inward as well as out of the pile. Therefore, in the wedge from near the low-tide line there was a second extended area colored by creosote. This area is designated by the letter C. It is less deeply colored than Zone B. Finally, the letter D on each of the wood samples designates the area that appeared to contain very little creosote.

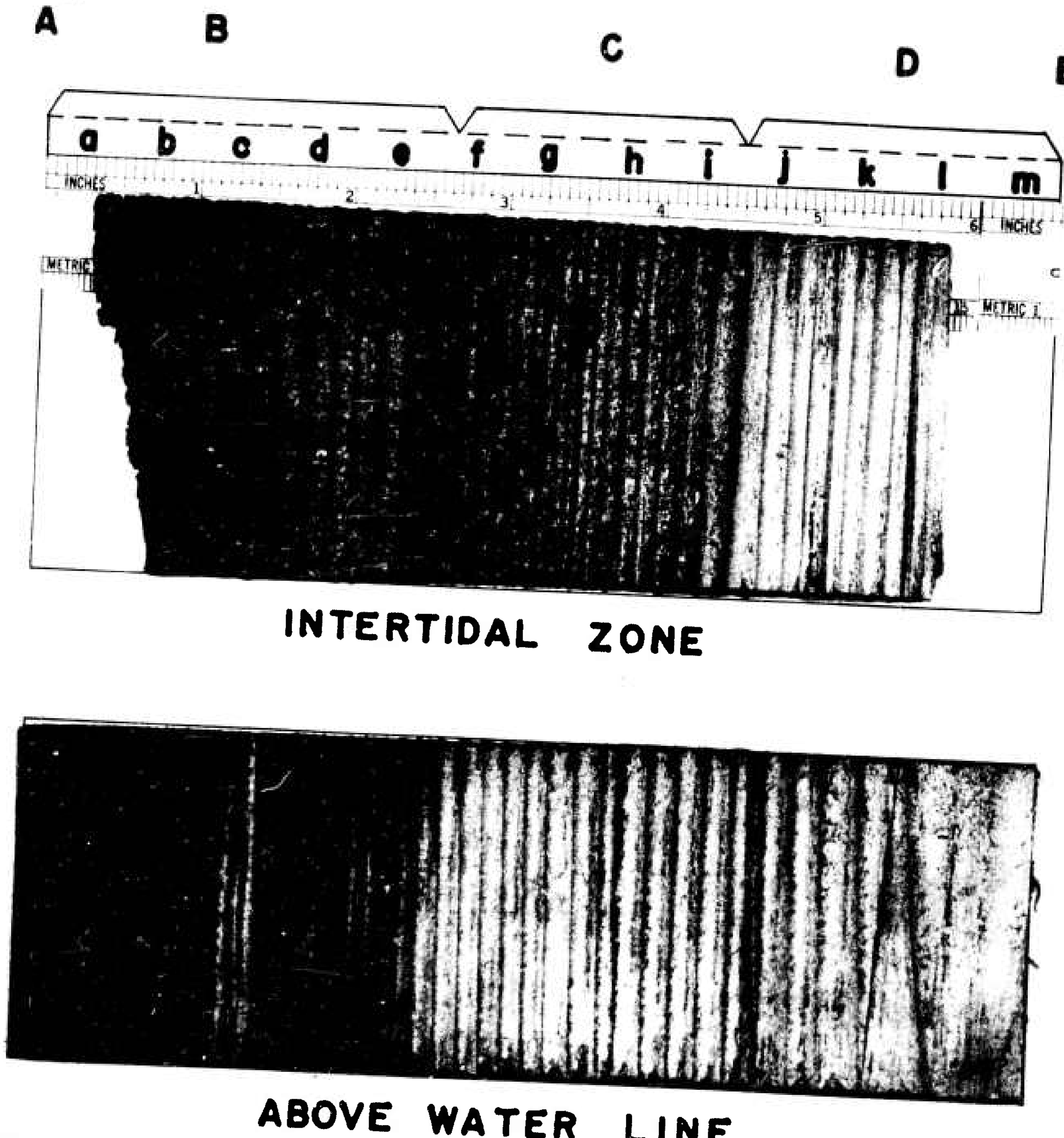

Figure 21. Creosote distribution in samples of wood removed from two regions of a creosoted pile.

A splinter was removed from each wedge at half-inch intervals from the outer edge of the center. The positions at which these splinters were removed are indicated by the lower case letters a through m (see Figure 21). Each splinter was placed in a separate Petri dish containing sea water and to these Petri dishes were added ten *Limnoria tripunctata*. Observations were made from time to time of the number of *Limnoria* still living. Figure 22 presents the results of this experiment graphically. It can be seen that the distribution of toxic material is more diffuse and extends deeper in the piling exposed to the leaching action of sea water. All but the outer 1/2-in. layer and the extreme inner region remain resistant to *Limnoria* even after exposure to the leaching action of sea water for thirteen years. The region of the wood containing the most toxic fractions of creosote are not in the layer originally impregnated.

Toxicity of Creosote Extracted from Various Parts of the Piling

Previous investigations had indicated that creosote or partially leached creosote was less toxic to *Limnoria tripunctata* than to *Limnoria quadripunctata*. Therefore the toxicities of whole creosote and leached creosote to both species were investigated.

Ten grams of a commercial brand of creosote were diluted to one liter with acetone. The small amount of carbonaceous material that would not dissolve was removed by filtration. Aliquots of the acetone filtrate were added to various volumes of sea water to prepare solutions or suspensions for toxicity tests.

Slabs of wood about one inch thick were removed from the exterior of a creosoted piling. The combined outer surface represented a little less than one square foot of piling surface. Extending inward from the outer surface for a distance of about one-half inch was a layer of wood heavily infested with *Limnoria*. This layer was thoroughly honeycombed with *Limnoria* burrows. This honeycombed portion of wood was scraped off with a large knife and collected. It represented wood susceptible to attack by *Limnoria tripunctata*. Previous tests had indicated that it was not susceptible to attack by *Limnoria quadripunctata*.

The material was air-dried and then extracted in a Soxhlet extractor for twenty-four hours with acetone. Finally it was extracted for another twenty-four hours with benzene. The extracted

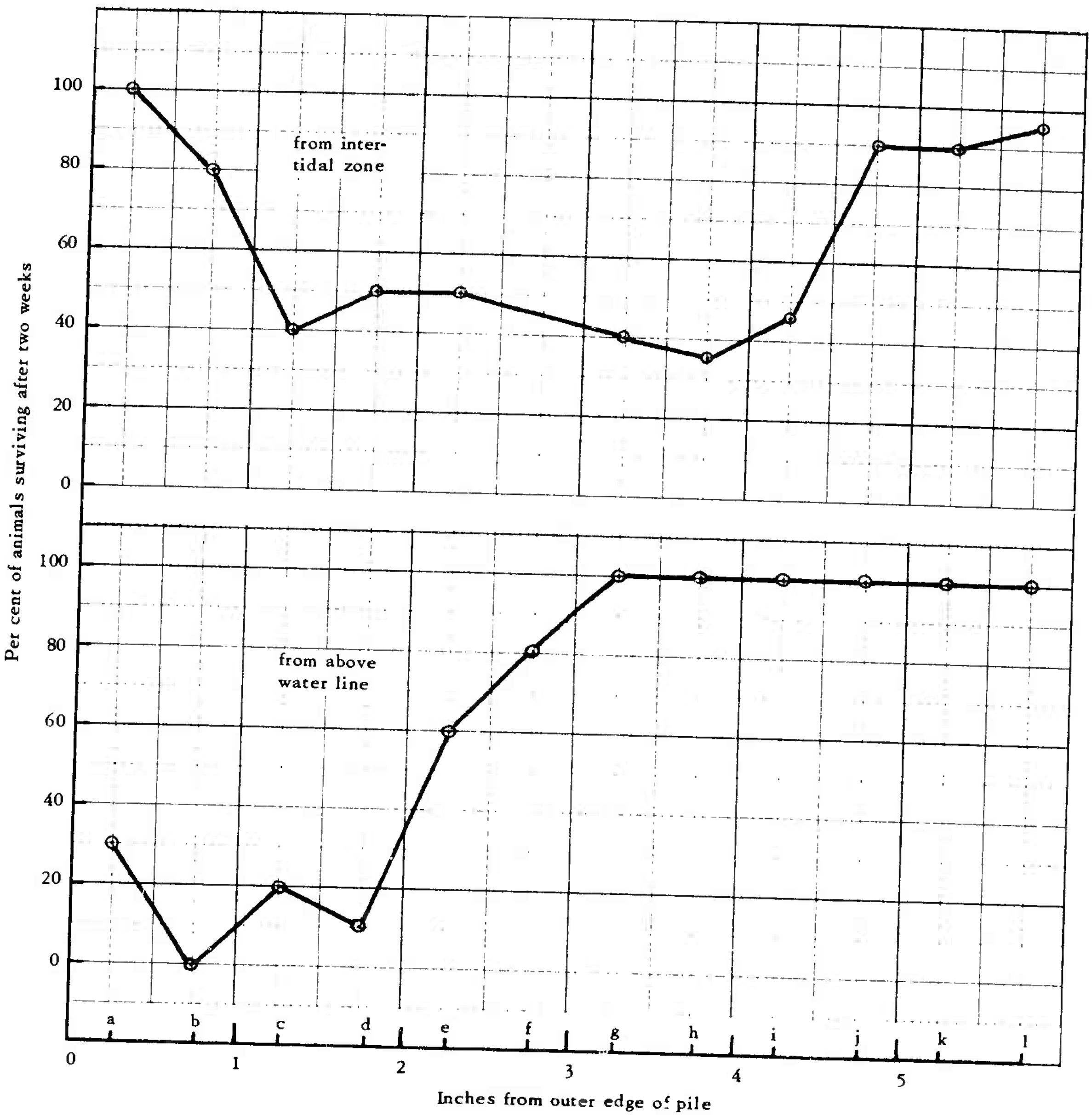

Figure 22. Per cent of *Limnoria tripunctata* surviving after feeding for two weeks on splinters removed at various depths into the interior of a leached creosoted piling.

material, consisting of wood and the skeletons of thousands of *Limnoria*, was then dried for three hours at 100 C. The weight of this dried material was 26.97 grams. The extracted wood was readily susceptible to attack by either *Limnoria tripunctata* or *Limnoria quadripunctata*.

Meanwhile the acetone and benzene extracts were combined and the readily volatile solvents were evaporated from the mixture. The residue was heated in an open dish for several hours on the steam bath. A tarlike material remained. It had only a slight odor of creosote. The weight of tarlike material thus obtained was 5.17 grams. An acetone solution of this was prepared in the same manner and concentration as had been used to prepare the commercial creosote standard. Aliquots of this were added to sea water to prepare solutions or emulsions for toxicity testing.

Next, the same slabs of wood from which the outer honeycombed area had been scraped, were cut into many small chips (about 1/8 in. by 1/8 in. by 1/2 in.). A number of these chips taken one or two inches inside the area of *Limnoria* infestation were dried at 110 C for three hours. They represented twelve cubic inches of piling and weighed 131 grams. Only 75 grams of this dried wood were employed in the experiment. The chips were extracted with acetone and benzene in the same manner as had been employed in extracting samples of wood taken from the area of *Limnoria* infestation. From it were obtained 14.9 grams of a tarlike substance having a fairly strong creosote odor. However, the odor was not as intense as that of creosote itself. The dry weight of the wood remaining after extraction was 50 grams. The apparent loss of 10 grams could only be explained as incomplete drying of the original material or loss of volatile materials.

The extracted wood was now susceptible to attack by *Limnoria tripunctata* and *Limnoria quadripunctata*. Solutions of the extracted tars in sea water were prepared in the usual manner.

Solutions or rather suspensions of the three creosote samples described in the proceeding paragraphs were prepared in concentrations of 100, 31.6, 10, 3.16, and 1.0 ppm by a serial dilution technique. Two dishes of each concentration of each sample were prepared. A small piece of a toothpick and ten *Limnoria* were added to each dish. The *Limnoria* added to one of the two sets of dishes were of the species *tripunctata* and to the other the species *quadripunctata*. The *Limnoria* *tripunctata* had been secured from the surface of a creosoted piling and were therefore designated creosote-tolerant *Limnoria*. The *Limnoria quadripunctata* were collected from a noncreosoted test block and were therefore called noncreosote-tolerant *Limnoria*.

At various intervals the number of animals surviving in each dish was observed. From the data an estimate was made of the concentration of each creosote sample required to kill 50 per cent of the animals in forty-eight hours.

Table III lists the results of the toxicity testing. Whole creosote was, in general, a little more toxic than the extract from the interior portions of the piling and considerably more toxic than the extract from the exterior of the wood. There was very little difference in the toxicity of whole creosote to *Limnoria tripunctata* and *Limnoria quadripunctata*. The creosote extracted from the interior of the pile was more toxic to *Limnoria quadripunctata* than to *Limnoria tripunctata*. However, the difference in toxicity was not great. The creosote extracted from the honeycombed exterior of the pile was essentially nontoxic to *Limnoria tripunctata* but was toxic to *Limnoria quadripunctata*. The toxicity to *Limnoria quadripunctata* was not great until the animals had been exposed for about one week.

CONCLUSIONS

Creosoted piling that had been removed from the harbor after ten to fifteen years of service was heavily infested with *Limnoria tripunctata*. The only other species of marine borers to be found in these timbers was an unidentified species of *Chelura*. The occurrence of the latter was not widespread.

The results of this study tend to confirm the postulate of Menzies that the species *Limnoria tripunctata* initiates the early failure of creosoted piling in California harbors and other southern ports. The results suggest that creosote or creosote additives employed to treat piling for use in these harbors should be chosen on the basis of ability to protect against *Limnoria tripunctata*.

Leaching by sea water tends to remove the most toxic ingredients of creosote from the outer layer of the piling. These more toxic ingredients are diffused both inward and outward by the action of the sea water.

TABLE III. Toxicity of several creosote fractions to *Limnoria quadripunctata* and *Limnoria tripunctata*.

Source of creosote	Dose required to kill 50% in 48 hours (ppm)	
	Limnoria tripunctata	*Limnoria quadripunctata*
Whole creosote	10	10
Extract from *Limnoria*-infested area of pile	> 100	50
Extract from non-infested area of pile	20	15

The ingredients of the creosote which are not readily leached by sea water apparently retain their toxicity to most species of marine borers but are relatively nontoxic to *Limnoria tripunctata*. Incorporation of a leach-resistant ingredient toxic to *Limnoria tripunctata* would greatly improve the usefulness of creosote.

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